Rapid Detection of Antimicrobial Susceptibility at the Point-of-Care

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Primary CNF Tools Used: Objet 3D printer

Abstract:

The rise of antibiotic resistant bacteria poses a serious threat to the United States. In 2014 President Obama issued an Executive Order and the White House published the National Strategy for Combating Antibiotic Resistance [1,2]. This document describes: how as antibiotic resistance continues to increase "we will no longer be able to reliably and rapidly treat bacterial infections", how "drug choices have become increasingly limited and more expensive and, in some cases, nonexistent", and imagines a world in which "modern medical advances such as surgery, transplants, and chemotherapy may no longer be viable due to the threat of infection." Already the Centers for Disease Control and Prevention reports that each year in the US, at least two million people acquire bacterial infections resistant to one or more antibiotics and at least 23,000 people die each year as a result [3]. In this project we are working towards developing a point-of-care diagnostic test that can rapidly provide antibiotic susceptibility information bloodborne organisms causing bacteremia in about four hours following a short blood culture. The integrated sample-to-answer system is based upon two key technologies already demonstrated in our labs: rapid blood sample clean-up using magnetic nano-beads, and a capillary-based system that enhances our ability to monitor bacterial metabolic activity, significantly reduces the time required to determine the efficacy of a given antibiotic dose.

Summary of Research:

In our initial experiments, we have adopted a colorimetric phenotypic testing approach to assess the antibiotic susceptibility of an attenuated *E. coli* K12 strain. The pH indicator phenol red was used to provide a phenotypic indication of bacterial growth.

For our colorimetric phenotypic testing approach, we conducted bacterial growth tests with small sample volumes in a PDMS microchip. The *E. coli* strain with a kanamycin resistance gene as selection marker was first streaked and incubated overnight on LB agar plates containing kanamycin. Single colonies were subsequently picked and propagated in liquid bulk culture. Following overnight bulk culture to stationary phase, small volumes (~1uL) of culture were separately incubated on the PDMS chip in wells each containing a 19 μL mixture of fresh LB media, 0.05% phenol red, and the antibiotics kanamycin (control) and ampicillin (test). The metabolic activity of viable bacteria leads to an accumulation of organic acids in the growth media, which causes the phenol red to change in color from red to yellow, which we expect for the control group as the *E. coli* stain is kanamycin resistant. Images of the chip were taken every hour, and a significant color change was detected after four hours, as seen in Figure 1.

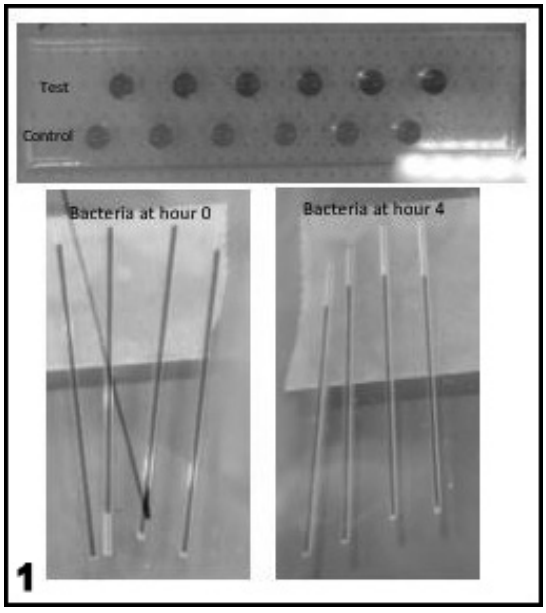


Figure 1: Color change over four hours.

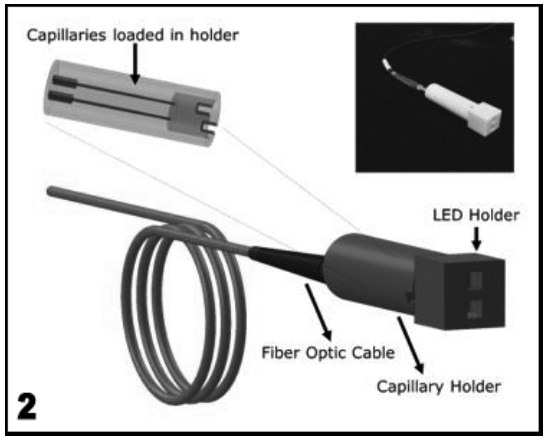
Assessing Bacteria Growth in a Capillary:

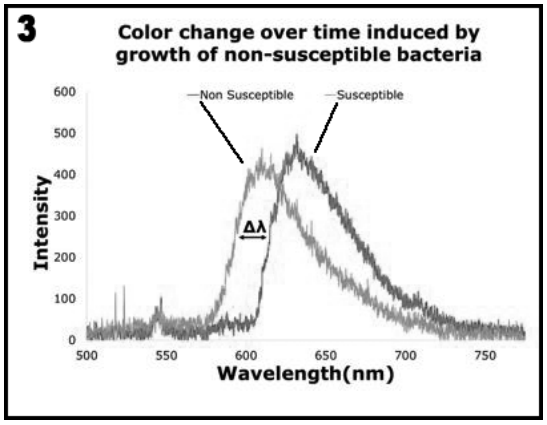
A similar experiment was conducted by incubating the bacteria culture in capillary tubes designed to hold microliters of liquid. We hypothesized that the increased surface area to volume ratio of the tubes would induce an accelerated growth rate for the bacteria. First, an overnight experiment was conducted to verify that incubation inside capillary tubes supported bacteria growth. Following this verification experiment, a shorter timescale experiment was conducted with added antibiotics. Similar to the previous experiment, a significant color change was detected in the control group (kanamycin) at the 4-hour mark, as shown in Figure 1. No color change was observed in the ampicillin inhibited group.

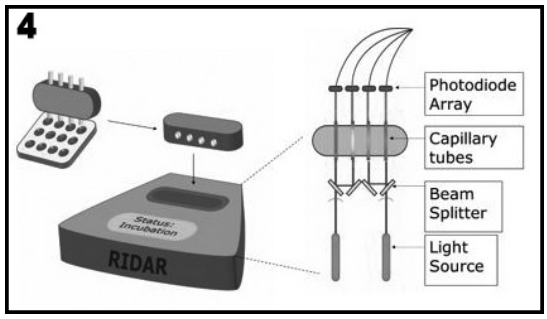
To perform real time detection of bacterial growth, we manufactured a small portable adapter using the Objet 3D printer at Cornell NanoScale Science and Technology Facility (CNF) (Figure 2). White light is incident on one end of the capillary tube and the spectra is measured on the other end using a small portable spectrometer (OceanOptics). A difference is output spectra can give us insight into whether the organism is inhibited by antibiotic presence. As seen in Figure 3, we are able to verify bacterial viability within six hours with an apparent shift in wavelength easily distinguishable by eye.

Future Considerations:

Further testing will include real time tracking of bacteria growth. In particular, we plan on devising a portable imaging system that will allow for real time measurements of the absorbance of phenol red, which we will use as a proxy for tracking bacteria growth. We envision a system as depicted in Figure 4, wherein a simple laser and photodiode system is used to measure absorbance across and along the capillary, thus allowing us to infer the growth of bacteria.







References:

[1] Report to the President on Combating Antibiotic Resistance - Executive Office of the President and President's Council of Advisors on Science and Technology. 2014; Available from:http://www.whitehouse.gov/sites/default/files/microsites/ostp/PCAST/pcast_carb_report_sept2014.pdf.

[2] National Strategy for Combatting Antibiotic Resistant Bacteria. 2014; Available from: http://www.whitehouse.gov/sites/default/files/docs/carb_national_strategy.pdf.

[3] Laxminarayan, R., A. Duse, C. Wattal, A.K.M. Zaidi, H.F.L. Wertheim, N. Sumpradit, E. Vlieghe, G.L. Hara, I.M. Gould, H. Goossens, C. Greko, A.D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A.Q. Peralta, F.N. Qamar, F. Mir, S. Kariuki, Z.A. Bhutta, A. Coates, R. Bergstrom, G.D. Wright, E.D. Brown, and O. Cars, Antibiotic resistance—the need for global solutions. The Lancet Infectious Diseases. 13(12): p. 1057-1098 (December 2013).